SOME CYTOECOLOGICAL ASPECTS IN THE EVOLUTION OF CERTAIN SPECIES OF THE PLANT GENUS *SILENE*[1]

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In this paper ecological, cytological, and genetical viewpoints are considered in analysis of the nature of the relationships between various species of the genus *Silene* and between closely related genera. In addition, the processes which are of evolutionary significance within the genus and between closely related genera are analyzed.

The genus *Silene* was first defined by Linnaeus in *Systema Naturae*, ed. 1, 1735, and *Genera Plantarum*, ed. 1, 1737. The genus was established in *Species Plantarum*, ed. 1, 1753, and as so delimited contained 26 species. The genus was revised superficially in 1824 by Otth in de Candole's *Prodromus* and extensively in 1868 by Rohrbach. The latest revision of the entire genus was made by Williams (1896) who built upon the work of Rohrbach and who enumerated 390 species in his monograph.. However, according to the records of Hitchcock and Maguire (1947) 200 more species have since been proposed. There is need of a further revision.

In North America the genus *Silene* was first monographed by Torrey and Gray (1838) who recognized 23 species; Robinson enumerated 45 species in his revision (1897); and Hitchcock and Maguire (1947), in the latest revision of the North American representatives of *Silene*, listed 54 species.

The genus *Silene* is widely distributed throughout the northern hemisphere and includes approximately 600 species, most of which occur in Europe, Asia, and northern Africa. Because the genus is exceedingly large and morphologically ill-defined, it is only with difficulty that it is separated from closely-related genera on morphological characters alone. In 1812 Rohling separated *Silene* from *Melandryum* (*Melandrium* of other authors) on the basis of the presence or absence of a partial septation in the capsule. Rohrbach (1868) accepted this generic character as did Williams (1896). If this character is used to limit the genus, many of the North American species and most of the species from the province of Yun-man would be transferred to the genus *Melandryum*. However, none of the American workers has recognized this criterion. Robinson points out that partial septation of the capsule is not a trustworthy character in American species because *Silene virginica* which is generally referred to *Melandryum* by continental authors often shows the partial septation of a "true" *Silene;* and *S. multinervia*, a "good" *Silene* by habits and affinity to others of the group, often has no trace of septation. This variable and intermediary condition found in most American species has led Hitchcock and Maguire to follow Robinson (1897) in not accepting American forms with unilocular capsules as representatives of a distinct genus, *Melandryum*.

Of the species studied in the present investigation, *Silene virginica* L., *S. rotundifolia* Nutt., *S. laciniata* Cav., and *S. regia* Sims are referred to the genus *Melandryum* by European workers; and *S. latifolia* Mill., *S. maritima* With., *S. stellata* L., and *S. fortunei* Vis. are classified as *Silene* by both groups of workers.

From this brief outline of the history of the taxonomic work on the genus *Silene*, it is evident that this genus must necessarily be approached from viewpoints other than morphological ones alone.

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METHODS AND PROCEDURES

Mature plants of *Silene virginica* and seed from *S. rotunidfolia* were collected in Hocking County, Ohio. Seed from *S. fortunei*, *S. laciniata*, *S. regia*, *S. stellata*, *S. maritima*, and *S. latifolia* were obtained from a commercial seed house. The seed was planted in December, 1948, and, following germination and growth, the plants began to flower in the spring of 1949. Various cross pollinations were made at this time to see which species were interfertile and to obtain hybrid material in which the interactions of the genomes of these species could be studied in various combinations. Cross pollinations of all possible combinations were not realized for the flowering periods of some pairs of species did not overlap. Not all of the cross pollinated species were cross fertile. Seed obtained from the successful crosses was planted in the fall of 1949 and the hybrid seedlings grew vigorously in each instance.

The fertility of these hybrids was tested in the spring of 1950 by making hand pollinations. The meiotic behavior of the chromosomes in both the parents and the F_1 individuals was observed on slides, each made by smearing the anthers of one bud in acetocarmine. Before smearing, the buds were fixed in a 3 : 1 absolute alcohol : acetic acid solution.

The photomicrographs were taken from permanent slides. The magnification of each of these photomicrographs is approximately 1400 except figure 2C, which is magnified approximately 330 times.

The geographic distributions of *S. maritima*, *S. latifolia*, *S. regia*, *S. laciniata*, and *S. fortunei* were plotted, based on data obtained from herbarium specimens obtained from New York Botanical Gardens, University of California at Los Angeles, West Virginia University, Brooklyn Botanic Gardens, the United States National Herbarium, and The Ohio State University.

THE PARENTAL SPECIES

The morphological characteristics which are used to separate the American species of *Silene* from the American species of *Melandryum* are variable and thus do not constitute a good basis for a natural system of classification. The base chromosome number of each of these genera is 12. Blackburn (1928) has reported the chromosome number for 45 species of *Silene* and 13 species of *Melandryum*. Of *Silene*, 41 species native to Europe, Asia, and North Africa, 1 species circumpolar in distribution, and 1 species native to North America have an *n* chromosome number of 12. The two other species of *Silene* are polyploid. Ten of the species of *Melandryum* native to the Old World have an *n* chromosome number of 12, while the 3 North American species have an *n* number of 24. Blackburn noted that the New World *Melandryum* species differed from the Old World species of the genus in that they were tetraploid. She stated that this difference was not paralleled in the genus *Silene* since *S. antirrhina*, the one New World species examined, has an *n* number of 12 as do most of the other widely-distributed species of this genus. In the present study the *n* chromosome number of a "true *Silene*," *S. stellata* L., var. *scabrella*, which is native to North America, was found to be 24 (fig. 2E). Thus, polyploids occur in the New World species of *Silene* as well as among the species of *Melandryum*, and American species, therefore, cannot be separated into these two genera on the basis of their chromosome numbers.

Blackburn reported that meiosis was regular in *S. latifolia* and *S. maritima* and that the *n* chromosome number of each of these Old World species is 12. *S. latifolia*, which has been introduced into North America, is a weed-like species that has spread throughout open fields, meadows, roadsides and forest borders from Maine, south to Virginia, southwest to Arkansas, and northwest to Washington and Oregon. *S. maritima* is confined to habitats along the sea coasts of England, Scotland, Ireland, Iceland, Norway, France, and Portugal. These

two species are reciprocally cross fertile (table 1), a finding in agreement with the work of Marsden-Jones and Turrill (1928). The resulting F_1 hybrids are fertile and seeds develop within the ovularies following open pollinations. No viable embryos develop following cross pollination between the diploid *S. maritima* or *S. latifolia* and the North American polyploid species *S. regia*, *S. rotundifolia*, or *S. laciniata*. *S. regia*, *S. rotundifolia*, *S. laciniata*, and *S. virginica* are cross fertile in all possible combinations (table 1). These polyploid North American species of *Silene* (considered species of *Melandryum* by European workers) are thus probably more closely related to one another than to the diploids *S. latifolia* and *S. maritima*. *Silene* is an exceedingly large and diversified genus, so it would not necessarily be expected that all members of this genus would be cross fertile. Williams (1896) noted that the members of the genus *Silene* "hybridize with difficulty," so it may well be that these two groups of species are merely rather distantly related species in *Silene*. Another factor to be considered is that cross fertilization might occur under other environmental conditions. The capsule septation basis for the separation of the North American species of *Silene* into two genera is of dubious value, both groups have the same basic chromosome number, and polyploids occur within both groups. The difference in the cross-

TABLE 1

*Results of Cross Pollinations of Silene Species Made During the Summers of 1949 and 1950.**

♂ / ♀	*fortunei*	*laciniata*	*latifolia*	*maritima*	*regia*	*rotundifolia*	*virginica*
fortunei	S	NCF	X	NCF	NCF	NCF	X
laciniata	X	S	X	X	S	S	S
latifolia	NCF	NCF	S	S	NCF	NCF	X
maritima	NCF	NCF	S	S	X	NCF	X
regia	X	X	X	X	S	S	S
rotundifolia	X	X	X	X	X	S	S
virginica	X	X	X	X	X	X	S

*X=no crosses attempted; S=seed obtained; NCF=no cross fertilization.

ability reported above, may be the result of the differences in the chromosome number of the species crossed. Because at the present time there is no evidence which makes it necessary to place these two groups in separate genera, it is suggested that they be considered members of one genus as the American workers have done in the past.

Silene fortunei Vis. is native to southeastern China and the Islands of Chusan and Formosa. Its habitats include grassy slopes, canyon sides, and river banks. This species, which has been classified as *Silene* by all who have worked with the genus, has an *n* chromosome number of 15 (fig. 1 C, D). *S. sinowatsoni*, another species native to China, has an *n* chromosome number of 12 (Blackburn, 1928). The base chromosome number for *Silene* is 12. Within this genus, none of the species which have been examined cytologically has an *n* chromosome number other than 12 or a whole multiple of 12. Not only is the chromosome number constant within this genus, but meiosis is an exceedingly regular process. It is thus difficult to even speculate upon a method by which an *n* number of 15 might originate within the genus unless it would be by intergeneric hybridization. However, there is no foundation for such a speculation. It is suggested that this species be transferred to another genus. Members of the genus *Vaccaria* and of the genus *Dianthus*, both of which are closely related to *Silene*, have a base number of 15. Because of the limited number of plants of this species examined, a sug-

gestion is not offered at this time as to whether it should be transferred to either of these two genera or to some other closely related genus or to a separate, new genus.

Of the four red-flowered species of *Silene* investigated, *S. regia* (fig. 1 E), *S. virginica* (Blackburn, 1928; Heaslip, 1950), and *S. rotundifolia* (Heaslip, 1950) are tetraploids and *S. laciniata* (fig. 2 D) is an octoploid. Meiosis is regular in each

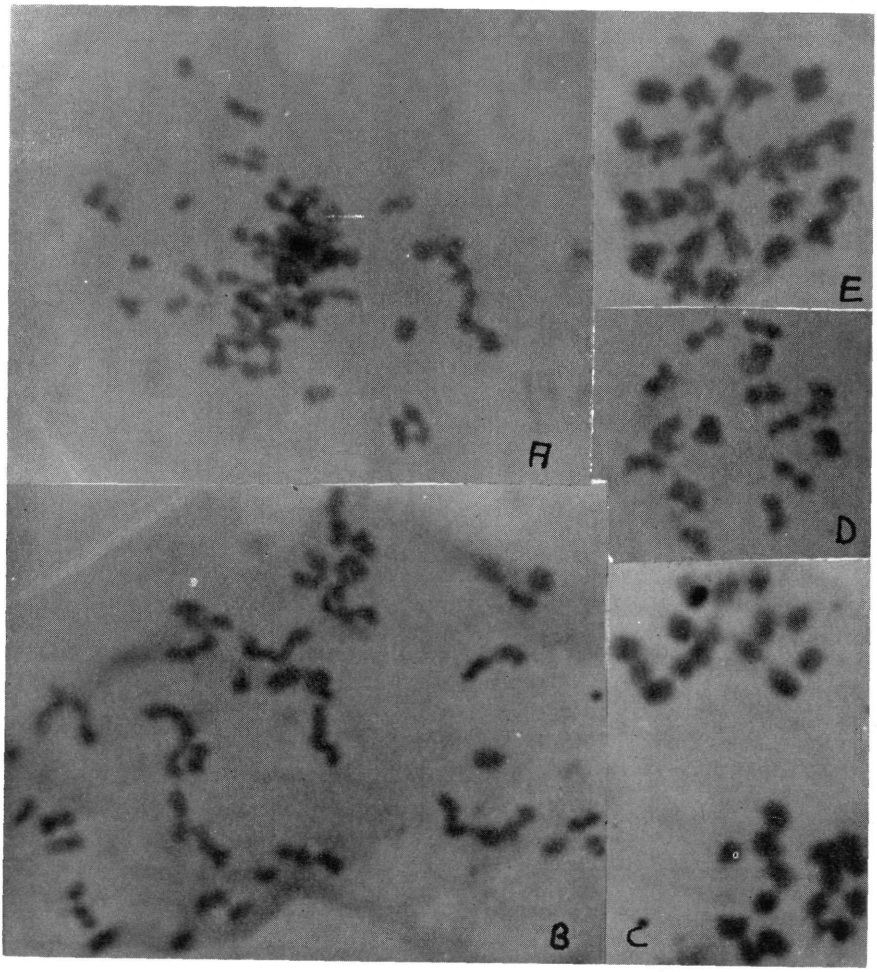

FIG. 1. (A) Hybrid of cross between *S. laciniata* and *S. rotundifolia:* metaphase I; pairing is irregular, note multivalents on the right side of the cell. (B) Hybrid of cross between *S. laciniata* and *S. rotundifolia:* polar view of early metaphase I; 1 quadrivalent, 5 trivalents, 17 bivalents, 19 univalents. (C) *S. fortunei:* anaphase I, 15 chromosomes at each pole of the spindle. (D) *S. fortunei:* metaphase I, 15 bivalents. (E) *S. regia*: metaphase II, polar view at one pole of the spindle. Photomicrographs by Dr. Joanne Parker.

of these species and no multivalents have been observed during meiosis. These species each appear to be completely self fertile. Because of this high fertility and the formation of bivalents only during the first meiotic division, it is probable that these species or their ancestors originated as amphiploids following interspecific hybridization rather than as autopolyploids.

INTERSPECIFIC HYBRIDS

It has not been possible to synthesize any of these red-flowered species but studies were made to determine some of the interrelationships among them by analyzing meiosis in and by self- and back-crossing the following F_1 hybrids:

S. rotundifolia ♀ × *S. virginica* ♂
S. laciniata ♀ × *S. rotundifolia* ♂
S. regia ♀ × *S. rotundifolia* ♂

Similar studies of the following F_1 hybrids were not possible for these plants were not in flower at the time of this report.

S. laciniata ♀ × *S. regia* ♂
S. laciniata ♀ × *S. virginica* ♂

Each of the F_1 hybrids investigated is vegetatively vigorous and has an extended flowering period. Although many of the flowers which occur on these hybrids are perfect, a large number of pistillate flowers occur on some plants. Stamens are formed in the pistillate flowers but they abort before the flowers open. The formation of either pistillate or perfect flowers is not correlated with length of day as it is in ragweed (Allard, 1945) for both types of flowers occur at the same time on the same plant. The occurrence of pistillate flowers is probably not entirely genetically controlled either, since both types of flowers sometimes occur within the same inflorescence. The amount of pollen formed in each anther of a hybrid flower is much less than in an anther of either parent, but the size and shape of all the pollen grains are similar. In the plants of each species used as parents the petals open, 5 stamens elongate, the anthers dehisce, and the other 5 stamens and the styles elongate or the other 5 stamens elongate followed by the elongation of the styles, and finally there is an expansion of the 3 stigmas. Pollination often occurs as the pistils grow to their final height. Because the stamens of the hybrid flowers sometimes never elongate, or the anthers do not dehisce until the pistils have grown above them, or the filaments collapse before the anthers dehisce, pollination often fails to occur. If pollination does occur by either natural or artificial means, the ovulary begins to enlarge and then dehisces when mature; but only shrunken, shriveled ovules occur within the capsule. Seed formed in the ovularies of self pollinated flowers on plants of each of the parent species at this time. Because all these groups of plants were subjected to the same environmental conditions, the hybrid sterility was not considered to be the result of an unfavorable environment.

Heaslip (1950) reported that the F_1 hybrid of the cross *S. rotundifolia* ♀ and *S. virginica* ♂ was both self and cross sterile. This sterility was attributed in part or wholly to meiotic irregularities such as failure of synapsis, formation of multivalents, lagging, and polyspory.

The F_1 hybrids of the cross *S. laciniata* ($n=48$) ♀ and *S. rotundifolia* ($n=24$) ♂ are basically hexaploids ($n=36$). This hybrid has visible characteristics of both parents. The stems are usually simple as in *S. laciniata*, freely branching, weak, decumbent as in *S. rotundifolia*, viscid-puberulent, as in both; the leaves are intermediate in size and shape between those of both parents, the cauline leaves being sessile, broadly lanceolate (twice as long as broad); basal leaves are more rounded, winged petiolate; inflorescence is open, flowers are more numerous than in either parent, more pubescent and viscid than other plant parts as in *S. laciniata;* corolla showy, color intermediate, the petals deeply bi-lobed, usually with an additional pair of smaller lateral lobes as in both parents.

Hundreds of the flowers on these hybrids were open pollinated and no seed formed, but this may have been the result of a failure of pollination. However, no seed formed following self- or back-crosses in which hand pollination was

practiced. *S. laciniata* and *S. rotundifolia* are thus closely enough related to be cross fertile; however, the vegetatively vigorous F_1 hybrid is sterile.

Many meiotic irregularities are observable during microsporogenesis in the anthers of this hybrid. Chromosome pairing is highly irregular and variable from cell to cell. Univalents, bivalents, and trivalents occur commonly (fig. 1 B);

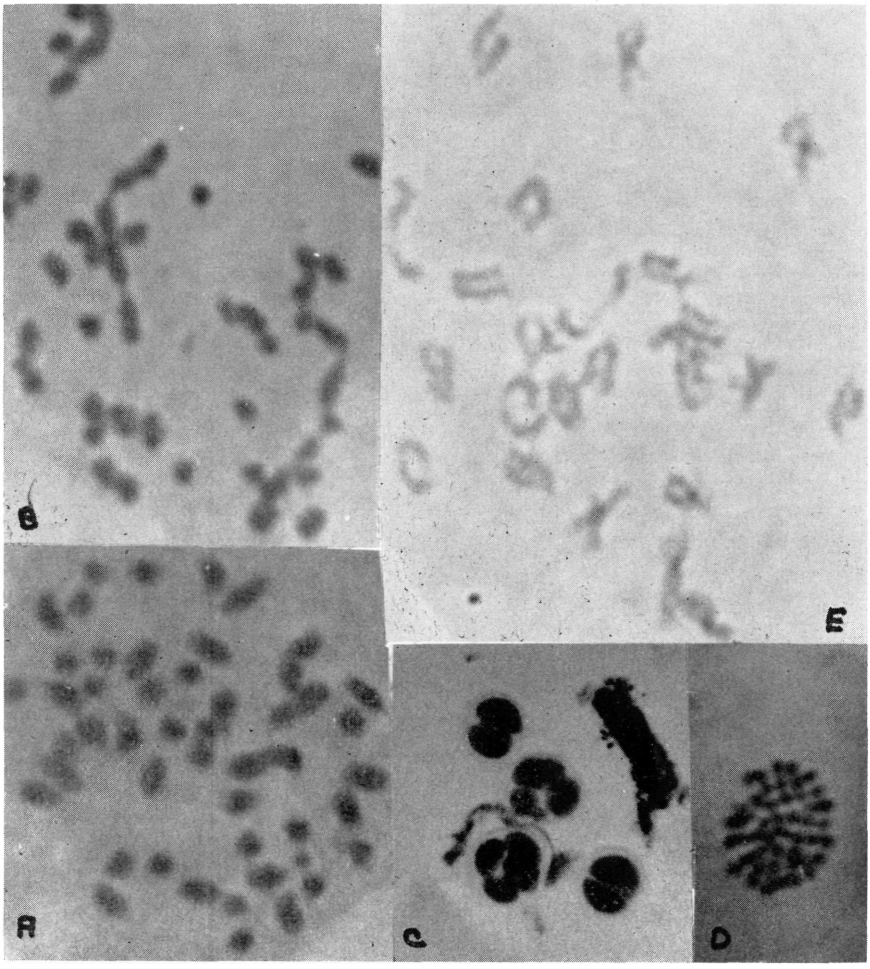

FIG. 2. (A) Hybrid of cross between *S. regia* and *S. rotundifolia:* metaphase I, most of the 48 chromosomes are present as univalents, no multivalents present. (B) Hybrid of cross between *S. regia* and *S. rotundifolia:* metaphase I, many multivalents present. (C) Hybrid of cross between *S. regia* and *S. rotundifolia:* polyspory. (D) *S. laciniata:* metaphase II, polar view at one pole of the spindle. (E) *S. stellata:* diakinesis, 24 bivalents. Photomicrographs A, B, C, D by Dr. Joanne Parker, E by Tillman Johnson.

quadrivalents and multivalents involving as many as 6 chromosomes occur occasionally (fig. 1 A). Numerical disjunction must not be regular for at anaphase I the number of chromosomes is not always equal in each of the polar groups. Such irregularities upset the genetic balance and may result in functional failure of the resulting gametes.

The chromosomes of each of the parents are so similar in size and shape that those of *S. laciniata* can not be distinguished from those of *S. rotundifolia* when they occur in the same cell of the hybrid. Therefore it is not possible to tell by observation whether allosyndesis or autosyndesis is occurring. Because trivalents, quadrivalents, and hexavalents do occur it is inferred that some of the chromosomes which synapse must come from the same parent. Autosyndesis does not occur in either parent but when chromosome sets of a polyploid are left without a partner, they frequently synapse audosyndetically (Darlington, 1937). The chromosomes from both parents are also involved in some of this pairing for multivalents involving more than 4 chromosomes occur. If synapsis can be used as an index for chromosome homology, then it can be inferred that *S. laciniata* and *S. rotundifolia*, which have chromosomes that synapse, are genetically closely related.

F_1 hybrids of the cross *S. regia* ♀ ($n=24$) and *S. rotundifolia* ♂ ($n=24$) are basically tetraploid. In these hybrids the stems are several, freely branching, weak, decumbent or ascending as in *S. rotundifolia;* leaves are intermediate between those of both parents, the cauline leaves are sessile, broadly lanceolate (once and a half as long as broad), lower leaves winged-petiolate; pedicels are densely viscid puberulent; calyx is densely viscid puberulent, tubular, becoming expanded by the maturing fruit as in *S. regia;* inflorescence is more pubescent and viscid than other parts of the plant; petals are shallowly bi-lobed and intermediate between those of both parents in color and shape.

These hybrids were in flower for approximately one month, but no seeds developed in any of the ovularies following hand pollinations. However, the ovularies increase greatly in size following pollination. Only back-crosses were made up to this report for the stamens in all the flowers which had opened had aborted. Both stamens and pistils abort in all of the flowers on some of these hybrids before anthesis.

Microsporogenesis in the anthers of this hybrid is irregular but pollen grains do form before the stamens abort. Synapsis is highly irregular and variable from cell to cell. At metaphase I in some cells, the greatest majority of the chromosomes are present as univalents (fig. 2 A), in others various multivalents occur (fig. 2 B). Instead of 4 microspores forming from each microspore mother cell, often more than 4 microspores, or 4 microspores and a variable number of micronuclei, form (fig. 2 C). Here again it is impossible to differentiate between autosyndesis and allosyndesis by observation alone but it can be inferred that the chromosomes of both parents are involved when multivalents involving more than 2 chromosomes occur. Since this synapsis does occur the best inference is that *S. rotundifolia* is genetically closely related to *S. regia.*

DISCUSSION

According to biosystematic criteria each of these 4 red-flowered species would be classified as an ecospecies for each has a separate genetic system that is balanced externally and internally, the genes of one of these species are not freely interchanged with those of another, and constitutional genetic barriers exist between them. All of these ecospecies would belong to the same cenospecies as there is a possibility of a limited interchange of genes from one species to another.

The relationship among these does not seem to be in a straight line nor does it appear to have a simple pattern. From the data at hand, it seems to be a complicated, reticulate pattern. *S. rotundifolia* has some chromosomes in common with *S. regia, S. virginica,* and *S. laciniata;* however the relationship is in no case great enough for the F_1 hybrid to be even partially fertile. No definite statements about the relationship of these four species can be made until the other hybrids are examined cytologically; however, some preliminary remarks can be made at the present time. The homology of some of the chromosomes of *S. rotundifolia* with the chromosomes of each of the other three species is an indication that these

species are interrelated through common ancestors. During speciation new chromosome combinations seem to have resulted from hybridization, apparently a series of hybridizations, for the synaptic homologies appear to be for parts of genomes rather than whole genomes.

Each of these species is now isolated from each of the others so that at the present time no introgression is taking place. *S. rotundifolia* has a limited distribution through southern Ohio, Tennessee, Kentucky, West Virginia, Virginia, and northern Alabama and Georgia (Wolfe, Wareham, and Scofield, 1949). Members of this species occur only on limestone or sandstone rock ledges or cliff crevices. However, the ecological amplitude of *S. virginica* appears to be much wider than that of *S. rotundifolia*. *S. virginica* occurs in open forests, thickets, or on rocky slopes from New Jersey, western New York, and southwestern Ontario, southward into Missouri and Georgia (Hitchcock and Maguire, 1947; Fernald, 1950). The distribution of *S. regia* occurs within the range of *S. virginica* and to the west of the range of *S. rotundifolia*. *S. regia* grows in open woodlands but it is primarily a prairie species which occurs from eastern Missouri and Arkansas, into southern Illinois and Ohio, Tennessee, Kentucky, and northern Alabama and Georgia. Although the range of distribution of *S. virginica* overlaps that of *S. rotundifolia* and *S. regia*, these species are ecologically isolated. Turrill (1938), in a study of the distribution of *Silene* in the French Alps, found that *S. alpina* and *S. cucubalus* were cross fertile but there was no amalgamating into a single polymorphic species because they are ecologically isolated. Not only are *S. rotundifolia*, *S. virginica*, and *S. regia* ecologically isolated but the sterility of the hybrids prevents these populations from becoming one species.

Hybrid sterility also is an effective barrier between *S. rotundifolia* and *S. laciniata*. *S. laciniata* subsp. *greggi* occurs on dry open slopes of southeastern Arizona through southern New Mexico into southwestern Texas, and south into Mexico. Thus *S. laciniata* is also geographically isolated from these other 3 species at the present time.

The difference between these species is probably not only the result of polyploidy and changes in the chromosome combinations, but gene mutations and reciprocal translocations have probably been important also. Because these morphologically distinct species have very similar karyotypes, it is inferred that gene mutations must have had a significant role in speciation. Evidence for the occurrence of reciprocal translocations was found in the F_1 hybrids of the cross between *S. rotundifolia* and *S. virginica* (Heaslip, 1950).

Within the genus as a whole, polyploidy does not seem to have played as important a role as it has in the speciation of North American members. Of all the Old World species of *Silene* examined but 4 are polyploids: *S. shafta* and *S. celliata* (Blackburn, 1928–1933), *S. pontica* and *S. vallesia* (Darlington and Ammal, 1945).

SUMMARY

1. At the present time, there is no evidence which makes it necessary to separate the American species of *Silene* into two genera; thus, it is suggested that the North American species referred to as *Melandryum* by European workers be classified as *Silene* as American workers have done in the past.

2. The *n* chromosome number of *S. regia* = 24, *S. stellata* = 24, *S. laciniata* = 48, *S. fortunei* = 15.

3. It is suggested that it may be best to transfer *S. fortunei* to another genus.

4. Although *S. rotundifolia*, *S. regia*, *S. virginica* are basically tetraploids and *S. laciniata* is basically octoploid, meiosis is regular in each of these species, indicating amphiploid rather than autopolyploid origin.

5. These 4 species are thought to be interrelated in a complex reticulate pattern.

6. These 4 species are cross fertile in all possible combinations and all the resulting hybrids are vegetatively vigorous.

7. Meiosis is irregular in the F_1 hybrids of the cross between *S. laciniata* ♀ ($n=48$) and *S. rotundifolia* ♂ ($n=24$), and in those of the cross between *S. regia* ♀ ($n=24$) and *S. rotundifolia* ♂ ($n=24$).

8. The sterility of these hybrids is attributed to the formation of genetically unbalanced gametes.

9. Polyploidy, interspecific hybridization, gene mutation, and reciprocal translocation are processes which have undoubtedly occurred in the evolution of these species of *Silene.*

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